Extraction and Isolation of Flavonoids Present in the Methanolic Extract of Leaves of *Acanthospermum Hispidium* Dc

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**ABSTRACT**

The leaves of *Acanthospermum hispidium* dc was extracted with polar and non-polar solvents. The active components (i.e. flavonoids) were found in methanol, chloroform, ethyl acetate and n-butanol while methanol, chloroform, ethylacetate and n-butanol contained steroids. From the chromatographic analysis, it was observed that the component 1and 2 have RF values of 0.61 and 0.48. The identification of the components and the specific absorption band were determined by spectroscopic analysis. In this paper we shall extract and isolate flavonoids present in the methanolic leaf extract of *Acanthospermum hispidium* DC

**Key words:** Acanthospermum hispidiumDC, Flavonoids, Methanolic Leaf

**Introduction**

Phytochemistry is the branch of chemistry that study the relationship between natural products and organic chemistry. The study of phytochemistry is very important and relevant because it helps to impact the knowledge of various plants constituent which can be tested for their pharmacological activity.

“Phyto” is a Greek word that means plant and phytochemicals are usually related to plant pigments. They are protective plant compounds. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties and they are produced by plant in order to protect it.

Phytochemicals such as flavonoids, saponins, tannins, glycosides e.t.c are not essential nutrients that are required by human body for sustaining life. There are many phytochemicals and each works differently. Most phytochemicals have anti-oxidant activity and protect cells against oxidative damage and reduce the risk of developing certain types of cancer.e.g. Flavonoids

Phytochemicals such as isoflavones found in soy have hormonal activity,i.e. It imitates human estrogen and helps to reduce menopausal symptoms and osteoporosis. Research suggested that phytochemicals, working together with nutrients found in fruits, vegetables and nuts, may help slow the aging process and reduce the risk of many diseases including cancer, heart diseases, stroke, high blood pressure, cataracts, and osteoporosis and urinary tract infections.

Phytochemicals can have complementary and overlapping mechanisms of action in the body including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system and modulation of hormone metabolism. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy, flavanoids in fruits, allylsulfides in onions, leeks and garlic, cartenoids in fruits and carrots and polyphenols in tea and grapes.

Saponins found in the beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin found in hot peppers, protects DNA from carcinogens and also allicin from garlic has anti-bacterial properties. The leaves of *Acanthospermumhispidium* dc have an ant malaria activity and it is used for the treatment of fever in Brazil.

**Natural Products:**

This section deals with the natural products

**Natural Products:**

Natural products are biochemical compounds that are isolated from living organisms [Plants, animals, insects]. Oil, carbohydrates, alkaloids, terpenoids, saponins, flavonoids are examples of natural products . They play important roles in both drug discovery and chemical biology because many approved therapeutics and drugs are derived from natural sources.

**Tannins:**

Tannins are widely distributed in plants. The name tannins are derived from their ability to tan leather and are not based on a class of compounds with a common basic structure. There are two groups of tannins;
The hydrolysable tannins, which are esters of Gallic acid and also glycosides of these esters.
The condensed tannins, which are polymers derived from various flavonoids.

Tannins are colourless non-crystalline substances which form colloidal solutions in water; these solutions have an astringent taste. It is found in leaf tissues, bud tissues, seed tissues, root tissues and stem tissues. It does not only heal burns and stop bleeding, but they also stop infection while they continue to heat the wound internally.

Tannins can also be effective in protecting the kidneys. They are also used for immediate relief of sore throats, diarrhoea, dysentery, hemorrhage, fatigue, skin ulcers. It has antiviral effects and is used to pull out poisons from poison oak or from bee stings, causing instant relief. It is also helps to draw out all irritants from the skin because it is an astringent that tightens pores and pulls out liquids.

Saponins:

Saponins are glycosides with a distinctive foaming characteristic. They are known as soap plants and are natural surfactants. They are found in many plants and act as active immune system.

Saponins are used as cough remedy and for diuretics. They act as a natural antibiotic and also bind cholesterol and thus interfere with cell growth and division. They are highly toxic to some creatures such as fish.

Steroids:

Steroids are lipids that are insoluble in water and can be extracted from cells by organic solvents of low polarity like ether or chloroform. Many plant steroids occur as glycosides and have the property of stimulating heart muscles. Steroidal glycosides have the property of forming foams in water (like soap solution) and so are known as saponins. Paper chromatography is a better means of separation of natural steroids while thin layer chromatography is useful for the identification of natural steroids. Example of steroid is cholesterol.

\[
\text{CH}_3 \quad \text{CH}_3
\]

STERIODS

Glycosides:

Glycosides contain a phenolic group and they occur in most parts of the plants. The simple glycosides are colourless, soluble in water and are optically active. They do not reduce Fehling’s solution.

\[
\text{ACO} \quad \text{OAC} \quad \text{OMe}
\]

GLYCOSIDES

On hydrolysis with inorganic acids, glycosides give a sugar and a hydroxylic compound, the aglycon, which may be an alcohol or a phenol. In natural state, each glycoside is usually associated with an enzyme which occurs in different cells of the plant.

Yan et al (2008) was able to isolate a new phenylpropanoid glycosides, 2-feruloyl-\(\alpha\)-\(D\)-glucopyranoyl-[1’ 2]-3,6-\(O\)-feruloyl-\(\beta\)-\(D\)-fructofuranoside from the root of Paris polyphylla var. yunnanensis. This plant is used for the treatment of liver, lung cancer and laryngeal carcinoma.
Cycloartane glycoside was isolated from the whole herbs of Camptosorus sibiricus by Yang et al (2006). The new-isolated constituent is 3β,7β,24β,25,30-pentahydroxycycloartane-24,30-di-O-β-D-glycopyranoside.

Kuang et al (2008) reported that a new phenolic glycoside, 3, 5-dimethyl-6-hydroxy-2-methoxy-4-O-D-glycopyranosyl-oxy-acetophenone was isolated from the aerial parts were used for the treatment of skin diseases, such as psoriasis, rash and dermatitis.

Alkaloids:

Originally, the name alkaloid (which means alkali-like) was given to all organic bases isolated from plants. Alkaloids can be defined as naturally occurring organic bases which contain a pyridine ring [Konigs, 1880]. It can also be defined as natural plant compounds having a basic character and containing at least one nitrogen atom in a heterocyclic ring [Lanburg, 1883]. Alkaloids are very poisonous but are used medicinally in a very small quantity.

Alkaloids are usually colourless, crystalline, non-volatile solids which are insoluble in water but are soluble in ethanol, ether, chloroform. e.t.c. Most alkaloids have a bitter taste and are optically active (laevorotatory). They have basic properties, complex structures, physiological action and are of plant origin. Alkaloids can be classified into eight (8) groups. They are:
1. Phenyl ethylamine groups. e.g. Tyramine

\[
\text{OH} \quad \text{CH}_2\text{CH}_2\text{NH}_2
\]

2. Pyrrolidine group. e.g. Hygrine

\[
\text{N} \quad \text{CH}_3 \quad \text{CH}_2\text{COCH}_3
\]

3. Pyridine and piperidine groups. e.g. Ricinine

\[
\text{Ome} \quad \text{N} \quad \text{CH}_3
\]

4. Pyrrolidine–pyridine group. e.g. Tropine

\[
\text{N} \quad \text{CH}_3 \quad \text{CH}_2\text{CH}_2\text{OH}
\]

5. Quinoline group. e.g. Galipine

\[
\text{N} \quad \text{CH}_2\text{CH}_2 \quad \text{OMe} \quad \text{OMe}
\]

6. Isoquinoline group. e.g. Papaverine

\[
\text{MeO} \quad \text{MeO} \quad \text{N} \quad \text{CH}_2 \quad \text{OMe} \quad \text{OMe}
\]

7. Phenanthrene group. e.g. morphine, codeine and thebaine
8. Indole group, e.g. Gramine

\[
\text{CH}_2\text{NMe}_2
\]

Awang et al (2008) reported that two new phenanthrene alkaloids, 2-hydroxyatherosperminine (A) and N-dimethyl-2-methoxyatherosperminine (B) can be isolated from the bark of *Cryptocarya crassinervia*.

\[\text{(A)}\]

\[\text{(B)}\]

Jin et al (2008) also isolated a new quinazolinedione alkaloid from the fruits of *Evodia officinalis*. The fruits of *E. officinalis* have long been used as a traditional Chinese drug in the treatment of headache, abdominal pain, dysentery, postpartum hemorrhage and amenorrhea.

**Flavonoids:**

Flavonoids are used to define all compounds whose structure is based on flavones. Thus the anthocyanins are one group of flavonoid compounds. They are natural plant pigments and are water-soluble, generally occur in the aqueous cell-sap and are responsible for the large variety of colours in flowers. These pigments are amphoteric; their acid salts are usually red, their metallic salts usually blue and in neutral solution, they are violet.

The isolation of these compounds depends on the plant source. The earlier methods used solvent extraction [ethanol, ether, acetone and light petroleum], but nowadays, chromatography is the main method.

Flavonoids can be classified into 6 major groups. They are;
1. Chalcones

2. Flavones (generally in herbaceous families e.g. Labiatae, Umbelliferae, compositae),

3. Apigenin (*Apium graveolens, Petroselinum crispum*), Luteolin (*Equisetum arvense*).

4. Flavonol (generally in woody angiosperms), Quercitol (*Rutagrarveolens, Fagopyrumesculentum, Sambucusnigra*), Kaempferol, myricetin.

5. Flavanone

6. Anthocyanins
Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. It also acts as powerful antioxidants. Ricardo et al (2004) have been able to isolate two flavonolglucosides [iso-quercitrin and hyperin] from the bioassay directed fractionation of the ethanolic extract of leaves of *Cryptocarya ashersoniana* seedlings and they discovered that the flavanol has antifungal and antiinflammatory activities.

Lin et al (2000) also isolated two new flavones glycosides with galloyl substitution from the dried fallen leaves of *Terminalia catappa L.* under the inhibition of Cu²⁺ induced LDL oxidation-guided fractionation. The two flavones glycosides are Apigenin 6-C-[2"-O-galloyl]-β-D-glucopyranoside and Apigenin 8-C-[2"-O-galloyl]-β-D-glucopyranoside and they showed significant anti-oxidative effects.

**FLAVONE GLYCOSIDE**

1. \( R1 = C-\beta-D-\text{glucosyl}[2^{-}\text{galloyl}], R2=R3=R4=H \)
2. \( R1=R3=R4=H, R2=C-\beta-D-\text{glucosyl } [ \text{galloyl} ] \)
3. \( R1=C-\beta-D-\text{glucosyl}, R2=R3=R4=H \)
4. \( R1=R3=R4=H, R2=C-\beta-D-\text{glucosyl} \)
5. \( R1=C-\beta-D-\text{glucosyl}, R2=R4=H, R3=OH \)
6. \( R1=R2=H, R3=OH, R4=O-\alpha-L-\text{rhamno [1"->6"] glucosyl} \)

Fang et al (2006) studied the rhizomes of *Cyclosorus acuminatus* and isolated six new flavanone glycosides from its methanol extract. Their structures were established on the basis of spectroscopic and chemical methods. These flavanone glycosides have anti-bacterial activities and they are:

a. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-glucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**

b. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-6-O-acetylglucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**

c. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-2,6-di-O-acetylglucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**

d. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-3,6-di-O-acetylglucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**

e. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-4,6-di-O-acetylglucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**

f. **(2s)-5-7-5'- trihydroxyflavanone 2'-O-β-D-3,4,6-tri-O-acetylglucopyranosyl-(1->3)-α-L-2-O-acetylrhamnopyanoside.**
Dried leaves of *Marchantia convoluta* are largely used as hepatoprotectives and to treat tumefaction of skins in China. Xiao et al. (2006) reported that two flavones [5-hydroxyl-7-methoxyl-2-methylchromone and Apigenin-7-O-β-D-glucuronide] can be isolated from the leaves of *Marchantia convoluta*. Several natural products [flavonols, terpenoids and steroids] were also identified as part of the chemical constituents of *Marchantia convoluta*.

Natural products are isolated in a pure form by techniques such as simple distillation, extraction with polar and non-polar solvent. The structure can be determined by spectroscopic techniques. Moon et al. (2005) also reported that natural products can be identified by the simple comparison of the nuclear magnetic resonance (NMR) data. The identification of natural products usually involve the separation of each compound and subsequent analysis of mass (MS) and NMR spectroscopy but for separation analysis, thin layer chromatography (TLC) and column chromatography can be used.

**Experimental Procedures:**

This section include the methods required in the isolation and characterization of organic compounds

**Sample Collection:**

The plant *Acanthospermum hispidium dc* was collected at Idi-Abebe in Ogbomosho, Oyo-State after sunset. The plant was identified by my project supervisor Mrs T.I Edewor.

**Preparation of the Sample:**

The leaves were air-dried after collection for two weeks in the laboratory condition for easy powdering. The dried leaves were ground into fine powder and then weighed.

**Extraction of the Sample:**

Solvent-solid extraction was carried out on the weighed, air-dried and pulverized leaves of *Acanthospermum hispidium dc*. The weighed sample was soaked with methanol for two days and the solvent was changed every twenty-four hours, until no extraction was observed. The separation of the residue from filtrate was done by using filter paper. It was followed by the concentration of the filtrate by using distillation method. The concentrated extract was weighed by using weighing balance.

Solvent-solvent extraction was also carried out. The weighed concentrated extract was suspended in the distilled water then extracted with n-hexane, chloroform, ethylacetate and n-butanol sequentially. The mixtures were shaken vigorously and were made to stand for some time for proper separation.

All the fractions gotten the extracts (i.e. n-hexane, chloroform, ethyl acetate and n-butanol fractions) were labeled properly and concentrated by distilling off the solvent using water bath.
Chromatography:

Two types of chromatographic methods were used to separate the constituents that were present in the leaves extract i.e. column chromatography and thin-layer chromatography.

Thin-Layer Chromatography (TLC):

TLC was used to ascertain the number of constituents present in the extract and to determine their purity. TLC was also used to determine the solvent mixture that will affect the separation of the components.

Preparation of Silica Gel Plates:

50g of silica gel powder was weighed into a conical flask, 100ml of distilled water was added and the resulting solution was shaken vigorously in order to avoid lumps.

The white smooth paste mixture was spread over the glass plate and was allowed to solidify. The coated glass plates were put inside oven for 1-2 hours at 110°C to ensure further solidification.

Spotting of the Plates:

This is done with aid of capillary tubes to introduce few drops of the dissolved sample extract unto the coated plate, allowing each drop to dry before adding another drop.

Developing of the Plates:

After the solvent had travelled some distance across the plate, the plate was removed and allowed to dry and then viewed in Iodine tank.

The separated components appeared as dark yellow spots in the Iodine vapour. The retention values were calculated by making use of the distance moved by the solvent and the distance moved by the component.

\[ RF = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}} \]

Column Chromatography:

This was done to isolate and purify the constituents present in the extracts.

Packing of Column:

- Dried glass column was held in place by retort stand and was sealed with glass-wool.
- The column was packed with n-hexane and silica gel as adsorbent and the column was tapped in order to avoid air-bubbles.
- 5ml of the extract was introduced into column then solvent mixture (eluent) in proper ratio was added into the column.
  
  Several fractions were obtained, concentrated and their purity was determined by using thin-layer chromatography. The impure fractions were further re-chromatography using a different solvent mixture.

Phytochemical Screening:

The following tests were carried out on crude extracts and solvent-solvent extracts (i.e. ethylacetate, n-butanol, chloroform and distilled water extracts) of *Acanthospermum hispidium* dc in order to ascertain the presence of these phytochemicals: flavonoids, tannins, glycosides, saponins, steroid and alkaloid.

- **Test for flavonoids:**
  
  1cm³ of 10% NaOH was added to 3cm³ of the extract. A yellow colouration indicates the presence of flavonoid.
• **Test for tannins:**

  1cm$^3$ of freshly prepared 10%KOH was added to the extract, a dirty white precipitate indicates the presence of tannins.

• **Test for glycosides:**

  To 1cm$^3$ of the extract in the test tube, 10cm$^3$ of 50%H$_2$SO$_4$ was added. The mixture was heated in boiling water for 15minutes. 10cm$^3$ of fehling’s solution was added, a brick red precipitate indicates the presence of glycosides.

• **Test for saponins:**

  Emulsion test: 5 drops of olive oil was added to 3cm$^3$ of the extract in a test tube, the mixture was vigorously shaken. A stable emulsion indicates the presence of saponins.

• **Test for steroid:**

  Salkowski test; 5 drops of concentrated H$_2$SO$_4$ was added to 1cm$^3$ of the extract. Red colouration indicates the presence of steroids in the extract.

• **Test for alkaloids:**

  To 1cm$^3$ of the extract, 2 drops of Maeyer’s reagent was added. A creamy precipitate indicates the presence of alkaloids in the extract.

**Spectroscopic Analysis:**

Infra-red (IR) analysis was carried out on the isolated compounds in order to determine their structure while Ultra-voilet (UV) was used to determine specific absorption band that were characteristic of isolated compounds.

**Results and Discussions**

*Results of the Crude Extract obtained from the Leaves of ACANTHOSPERMUM HISPIDIUM DC:*

Weight of the powdered sample = 1000g

Weight of the dried beaker = 174.5g

Weight of beaker + sample extract = 272.8g

Weight of the extract sample = 98.3g

%Yield of extract = \( \frac{\text{weight of extract}}{\text{weight of powdered sample}} \times 100 \)

Weight of powdered sample

= 98.3 \( \times 100 \)

1000

= 9.83%

*Result of Phytochemical Screenings:*

<table>
<thead>
<tr>
<th>Table 1: Phytochemical screening of the crude extract.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extracts</strong></td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
<td>N-hexane</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Ethylacetate</td>
</tr>
<tr>
<td>N-butanol</td>
</tr>
<tr>
<td>Water</td>
</tr>
</tbody>
</table>
Table 2: Percentage weight of fraction with their colours.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%Weight</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>50.67</td>
<td>Black</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.31</td>
<td>Green</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>5.27</td>
<td>Yellow</td>
</tr>
<tr>
<td>n-butanol</td>
<td>4.62</td>
<td>Gold</td>
</tr>
<tr>
<td>Water</td>
<td>37.13</td>
<td>Brick red</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Phytochemical test on fractionated extracts.

<table>
<thead>
<tr>
<th>TEST</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. N-hexane extract + 10%NaOH</td>
<td>No yellow colouration</td>
<td>Flavonones absent.</td>
</tr>
<tr>
<td>N-hexane extract + conc. H₂SO₄</td>
<td>No orange colouration</td>
<td>Steroid absent.</td>
</tr>
<tr>
<td>2. Chloroform extract + 10%NaOH</td>
<td>Yellow colouration</td>
<td>Flavonones present.</td>
</tr>
<tr>
<td>Chloroform extract + conc. H₂SO₄</td>
<td>Orange colouration</td>
<td>Steroid present.</td>
</tr>
<tr>
<td>3. Ethyl acetate extract + 10%NaOH</td>
<td>Yellow to orange</td>
<td>Isoflavones present.</td>
</tr>
<tr>
<td>Ethylacetate extract + conc.H₂SO₄</td>
<td>Yellow to orange</td>
<td>Steroid present.</td>
</tr>
<tr>
<td>4. N-butanol extract + 10%NaOH</td>
<td>Yellow to orange</td>
<td>Flavones present.</td>
</tr>
<tr>
<td>N-butanol extract + conc. H₂SO₄</td>
<td>Orange to crimson</td>
<td>Steroid present.</td>
</tr>
<tr>
<td>5. Water extract + 10% NaOH</td>
<td>Yellow to orange</td>
<td>Flavonols and flavones present.</td>
</tr>
<tr>
<td>Water extract + conc. H₂SO₄</td>
<td>Yellow to orange</td>
<td>Flavanols and flavones present.</td>
</tr>
</tbody>
</table>

Table 4: Test for class of flavonoids.

<table>
<thead>
<tr>
<th>TEST</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Chloroform extract + aqueous NaOH</td>
<td>Yellow</td>
<td>Present</td>
</tr>
<tr>
<td>B. Ethylacetate extract + Aqueous NaOH</td>
<td>Yellow to orange</td>
<td>Present</td>
</tr>
<tr>
<td>C. N-butanol extract + aqueous NaOH</td>
<td>Yellow</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Result from Chromatographic and Spectroscopic Analysis:

Retention factor which is the distance moved through the stationary phase to that of mobile phase. Column chromatography gave different fractions and these fractions were concentrated and their purity was determined by using thin- layer chromatography. The fractions obtained and their R_f values as follows:

Table 5: R_f values and weight % for isolated compounds.

<table>
<thead>
<tr>
<th>FRACTIONS</th>
<th>R_f VALUES</th>
<th>%WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component 1</td>
<td>0.61</td>
<td>0.08</td>
</tr>
<tr>
<td>Component 2</td>
<td>0.48</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 6: IR DATA OF COMPOUND 1.

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>PROBABLE ASSIGNMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2988.30</td>
<td>C-C stretch</td>
</tr>
<tr>
<td>1772.53</td>
<td>5 membered ring lactones</td>
</tr>
<tr>
<td>1646.35</td>
<td>C-O stretch</td>
</tr>
<tr>
<td>1042.64</td>
<td>C-O stretch</td>
</tr>
<tr>
<td>1436.38</td>
<td>C-CH₃ bend vibration</td>
</tr>
</tbody>
</table>

Table 7: IR DATA OF COMPOUND 2.

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>PROBABLE ASSIGNMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3450.21</td>
<td>Phenolic OH stretch</td>
</tr>
<tr>
<td>2936.45</td>
<td>C-H stretching vibration</td>
</tr>
<tr>
<td>1772.19</td>
<td>5 membered ring lactone</td>
</tr>
<tr>
<td>1636.91</td>
<td>C=C stretch</td>
</tr>
<tr>
<td>1042.54</td>
<td>C-O stretch</td>
</tr>
</tbody>
</table>
Discussion:

A kilogramme of powdered sample of *Acanthospermum hispidium* dc was soaked with methanol for two days, 98.3g extract was obtained. Phytochemicals screening was carried out on methanol, n-hexane, trichloromethane, ethylacetate, n-butanol and water extracts. The presence of steroid and flavonoid were discovered in methanol, trichloromethane, ethylacetate and n-butanol extracts while saponins, tannins, alkaloid and glycosides were absence in the extracts. Flavonoid was also discovered in water extracts and no phytochemical was found in n-hexane.

The isolation and purification of the components were done by using column chromatography and thin-layer chromatography while infra-red analysis was carried out for the identification of the components and the specific absorption band was determined by ultra-violet analysis. Component 1 and component 2 are flavonoids but because of incomplete spectroscopic data, the structures cannot be determined.

Conclusion:

The leaves of *Acanthospermum hispidium* dc contain steroids and flavonoids (i.e. flavanones, isoflavones, flavones and flavonols). It can therefore be used to treat tuberculosis and also it can acts as an anti-poisoning agent.

References

Mabry, T.J., K.R. Markham, M.B. Thomas, 1970. The systematic identification of flavonoids, “SSpringer-
and flavanoids from Cryptocaryaashersoniana seedlings, ARKIVOC, 6: 127-136.
sciences, 3(1): 310-313.
Yamamoto and Gaynor, 2006. Therapeutic potential of inhibition of the NF-KB pathway in the trearment of
Zang,W.M., 2008. A new phenolic glycoside from the aerial parts of Dryopterisfragrans,Fitoterapia, 79: 319-
320.